

Two new species of *Oswaldocruzia* (Nematoda: Trichostrongylina: Molineoidea) parasites of the cane toad *Rhinella marina* (Amphibia: Anura) from Peru

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Abstract

Two new species of *Oswaldocruzia*, *O. manuensis* sp. nov., and *O. urubambaensis* sp. nov. are described and illustrated from Peru, these are parasites of the cane toad *Rhinella marina*. *O. manuensis* is characterized by having cervical alae which are not well developed, ridges without chitinous supports, caudal bursa type II and branches of fork of dissimilar length. *O. urubambaensis* is characterized by a caudal bursa of type I, ridges with chitinous supports, a thin cephalic vesicle and origin of rays 9 in tip of the dorsal trunk.

Keywords

Trichostrongylids, parasites, new species, cane toad, Peru

Introduction

The genus *Oswaldocruzia* Travassos, 1917 is cosmopolitan and comprises 88 species (Moravec and Kaiser 1995; Ben Slimane, Durette-Desset and Chabaud 1996; Bursey and Goldberg 2004, 2005, 2011; Bursey *et al.* 2006, 2007; Durette-Desset *et al.* 2006; Santos *et al.* 2008) of parasites found mostly in toads and frogs but also in salamanders, lizards and rarely in snakes. There are 39 known species in the Neotropical region, 30 in South America and 9 in Caribbean Islands, 36 species are parasitic in Bufonidae around the world and 6 in *Rhinella marina*, but it is a group actively evolving and some not yet described species would be found. Parasites collected in cane toads from Peru revealed two unnamed species described as follow.

Materials and Methods

Live cane toad were found in tropical rain forest and collected manually in, Manu National Park, Cusco, Peru, in February 1992 and in May 1997. To euthanize the host were submerged in solution of chlorotone following the protocol of McDiarmid (1994). The parasites were collected from host's gut, preserved in ethanol (70%), and clarified in lactophenol. All measurements are in µm unless otherwise stated and given as the first

number corresponds to the mean, the numbers in parentheses to the extremes. Illustrations were made using a Nikon microscope with the aid of the camera lucida. The synlophe and the spicules are described according to Ben Slimane, Durette-Desset and Chabaud (1996). Host names follow Frost (2011). Types are deposited in the Colección de Parasitología, Museo de Biología, Universidad Central de Venezuela (Venezuela) (CP-MBUCV), in the National Collection of Zooparasitic Nematodes of the Muséum National d'Histoire Naturelle of Paris (MNHN) (France), and Colección de Helmintología of the Universidad Nacional Mayor de San Marcos of Lima (Peru) (CH-UNMSM).

Results

Oswaldocruzia manuensis sp. nov. (Fig. 1)

General: Small size nematodes, not coiled. Cephalic vesicle present with light anterior swelling. En face buccal aperture triangular prolonged ventrally, with 6 externo-labial papillae, 2 amphids and 4 cephalic papillae. Dorsal oesophageal tooth developed. Subtriangular-shaped deirids, posterior to excretory pore. Well developed excretory glands. Musculo-glandular oesophagus separation clearly visible at nerve ring level. Cervi-

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cal alae present but short, not reaching oesophagus end. Cuticular ridges without chitinous support in both, females and males. Absence of ridges opposite lateral hypodermic cords.

Synopse: In both sexes, cuticle bears uninterrupted longitudinal ridges without chitinous support while appear posterior the cephalic vesicle and disappear just anterior to caudal bursa in males and at phasmids level in females.

Cervical alae appear at 30–50 posterior cephalic vesicle and is composed of one triangular and rounded crest lightly

oriented toward ventral side in males and same but less developed in females.

In males 19–21 ridges (10–12 dorsal, 7 ventral, 2 alae) at nerve ring level; 22–26 ridges (12–14 dorsal, 8–10 ventral, 2 alae) at excretory pore level, 29–31 (15–16 dorsal, 12–13 ventral, 2 alae) at oesophago-intestinal junction, 39 (20 dorsal, 19 ventral) at mid-body, all without chitinous supports. In females 27 ridges (16 dorsal, 9 ventral, 2 alae) at nerve ring level, 27–32 (12–18 dorsal, 10–13 ventral, 2 alae) at excretory pore level,

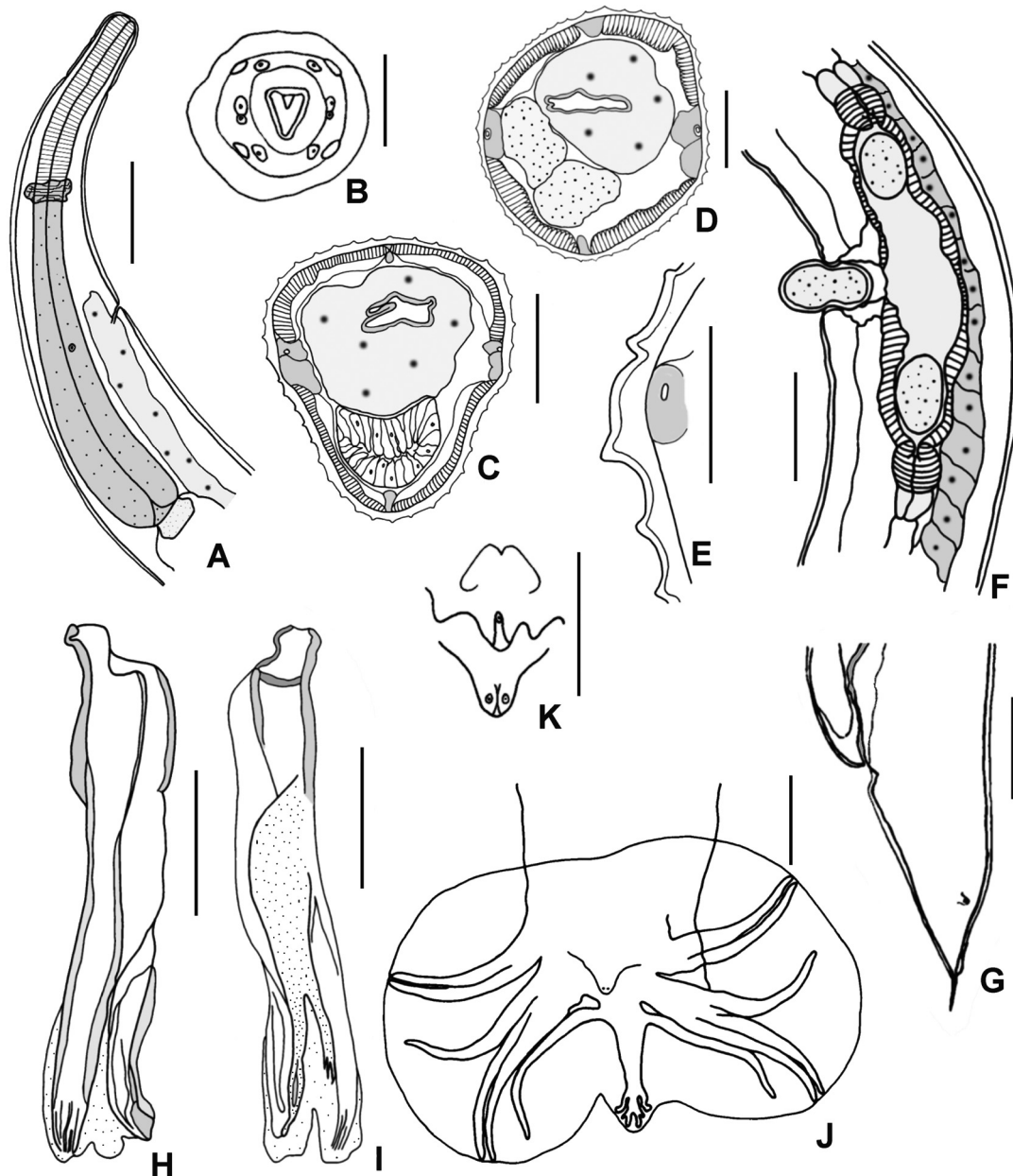


Fig. 1. A-K. *Oswaldocruzia manuensis* sp. nov. **A.** Female, anterior end, right lateral view; **B.** Female, head apical view; **C.** Female, transverse section at mid-body; **D.** Male, transverse section at mid-body; **E.** Female, right cervical ala at level of excretory pore; **F.** Female, vulvar and ovejector regions, left lateral view; **G.** Female, tail, left lateral view; **H.** Male, left spicule, externo-dorsal view; **I.** Male, left spicule, ventral view; **J.** Male, caudal bursal, ventral view; **K.** Male, genital cone, ventral view. Scale bars: A, F-G = 100 µm; B, E, M = 20 µm; C, D, H-J = 50 µm

34–37 (18–22 dorsal, 14–15 ventral, 2 alae) at oesophago-intestinal junction, and 54 (27 dorsal, 27 ventral) at mid-body all crests oriented perpendicularly to body surface, spaced regularly, of the same size, and without chitinous support.

Male: (14 exemplars) 6.3 (4.3–9.1) mm long and 158 (132–184) wide close to mid-body. Cephalic vesicle 75 (66–85) long and 39 (34–44) wide. Nerve ring, excretory pore and deirids 188 (170–213), 306 (214–367), and 340 (251–419) from apex, respectively. Oesophagus 484 (388–553) long.

Caudal bursa with pattern of type 2–3 which tends towards type 2–1–2, so extremities of rays 4 curved towards couple 2–3 then papillae of rays 4 situated closer to papillae 3 than 5. Rays 8 arising perpendicularly on root of dorsal ray and lightly overlapped by rays 6 (Type II). Rays 9 arising distally on trunk of dorsal ray anterior division of latter into two branches (rays 10). Gubernaculum absent. Genital cone 14–18 long and 24–28 wide at base, bearing very large papilla zero on anterior lip and 2 less developed papillae on posterior lip.

Spicules 195 (180–208) long, divided proximally, into three main branches, shoe, blade (distally divided into 5–7 small branches of unequal length), and fork divided at 19–27.2% of total length of spicule.

Female: (18 exemplars) 9.1 (5.4–14.2) mm long and 161 (137–199) wide at mid-body. Cephalic vesicle 78 (67–90) long and 42 (34–44) wide. Nerve ring, excretory pore, and deirids situated at 200 (175–235), 315 (248–439), and 341 (274–456) from apex, respectively. Oesophagus 490 (401–564) long.

Didelphic, vulva at 2.9 (1.7–4.3) mm from caudal extremity. Vagina vera: 52 (48–62) long, dividing vestibule into two parts, anterior 183 (154–247) long and posterior 158 (120–180) long. Sphincters both 39 (34–44) and infundibula both 42 (33–50) long, respectively. Uterine branches with 42–84 eggs. All eggs at morula stage, 81 (73–91) long and 43 (38–46) wide. Tail 170 (110–229) long and 74 (60–94) wide at anus level with caudal spine 14 (12–20) long. Phasmids at 48 (34–62) from posterior tip.

Host: *Rhinella marina* (Linnaeus, 1758) (Bufonidae).

Location: small intestine.

Locality: Pakitza, Parque Nacional Manu, Madre de Dios, Peru (11°56'S, 71°15'W, 360 m).

Types: Holotype male (CH-UNMSM 1535), allotype female (CH-UNMSM 1536), paratypes 5 males and 9 females (CH-UNMSM 1537), 2 males and 3 females (MNHN 645 KQ), host N° 178-100292.

Other material 6 males and 10 females (CP-MBUCV 6105) same data of types, but host N° 47-100292.

Etymology: Toponymic derived from the type locality.

Discussion: The species belong to the Neotropical Continental Group of Ben Slimane, Durette-Desset and Chabaud (1996) and is closer to *Oswaldocruzia* with cervical alae, ridges without chitinous support and caudal bursa type II, three species have these characteristics: *O. vaucheri* Ben Slimane et Durette-Desset, 1993 parasite of Leptodactylidae from Ecuador, *O. peruensis* Ben Slimane, Verhaagh et Durette-Desset, 1995 a parasite of Iguanidae from Peru and *O. venezue-*

lensis Ben Slimane, Guerrero et Durette-Desset, 1996 parasite of Bufonidae from Venezuela. *O. vaucheri* and *O. peruensis* have outgoing and well-developed cervical alae with rays 8 not perpendicular to dorsal ray, but in *O. manuensis* sp. nov. the cervical alae are not well developed and rays 8 arises perpendicularly to dorsal ray same as in *O. venezuelensis* but in latter papillae of rays 4 situated at about same distance between papillae of rays 3 and 5 and branches of fork have same length, in *O. manuensis* sp. nov. papillae of rays 4 is situated closer to papillae 3 than 5, and branches of fork are of dissimilar length.

Oswaldocruzia urubambaensis sp. nov. (Fig. 2)

General: Medium size nematodes, uncoiled. Cephalic vesicle present with very light anterior swelling. En face buccal aperture oval, with 6 externolabial papillae, 2 amphids and 4 cephalic papillae. Dorsal oesophageal tooth well developed. Deirids longer than wide, and posterior to excretory pore. Well-developed excretory glands. Musculo-glandular oesophagus separation clearly visible at nerve ring level. Cervical alae present but short, not reaching oesophagus end. Cuticular ridges with chitinous support in both, females and males. Without ridges opposite lateral hypodermic cords.

Synopse: In both sexes, cuticle bears uninterrupted longitudinal ridges which appear posterior the cephalic vesicle and disappear just anterior to caudal bursa in males, and at phasmids level in females. Cervical alae appear posterior to cephalic vesicle and are composed of one triangular crest lightly oriented towards ventral side.

In males 26 ridges (14 dorsal, 10 ventral, 2 alae) at nerve ring level; 28–31 ridges (13–16 dorsal, 10–13 ventral, 2 alae) at excretory pore level, 34–35 (18–19 dorsal, 16–16 ventral) at oesophago-intestinal junction, 44 (23 dorsal, 21 ventral) at mid-body, all with chitinous support. In females 27 ridges (15 dorsal, 10 ventral, 2 alae) at nerve ring level, 29–30 (15–17 dorsal, 11–12 ventral, 2 alae) at excretory pore level, 35–36 (18–21 dorsal, 15–17 ventral) at oesophago-intestinal junction, 50–60 (26–30 dorsal, 24–30 ventral) at mid-body, and 58–61 (55–36 dorsal, 22–25 ventral) at vestibule level, all crests oriented perpendicularly to body surface, spaced regularly, same size, and with chitinous support.

Male: (13 exemplars) 9.0 (6.9–10.0) mm long and 174 (149–199) wide close to mid-body. Cephalic vesicle 79 (70–89) long and 46 (41–53) wide. Nerve ring, excretory pore, and deirids 208 (173–230), 341 (271–425), and 379 (307–472) from apex, respectively. Oesophagus 524 (475–578) long.

Caudal bursa with pattern of type 2–3 which tends towards 2–1–2, so extremities of rays 4 curved towards couple 2–3 then papillae of rays 4 situated closer papillae 3 than 5. Rays 8 arising perpendicular on root of dorsal ray and parallel or lightly overlapped by rays 6 (Type I). Rays 9 arising distally on trunk of dorsal ray well anterior to division of the latter into two branches. Gubernaculum absent. Genital cone 46 long and 43 wide at its base, with 2 slight developed papillae on posterior lip.

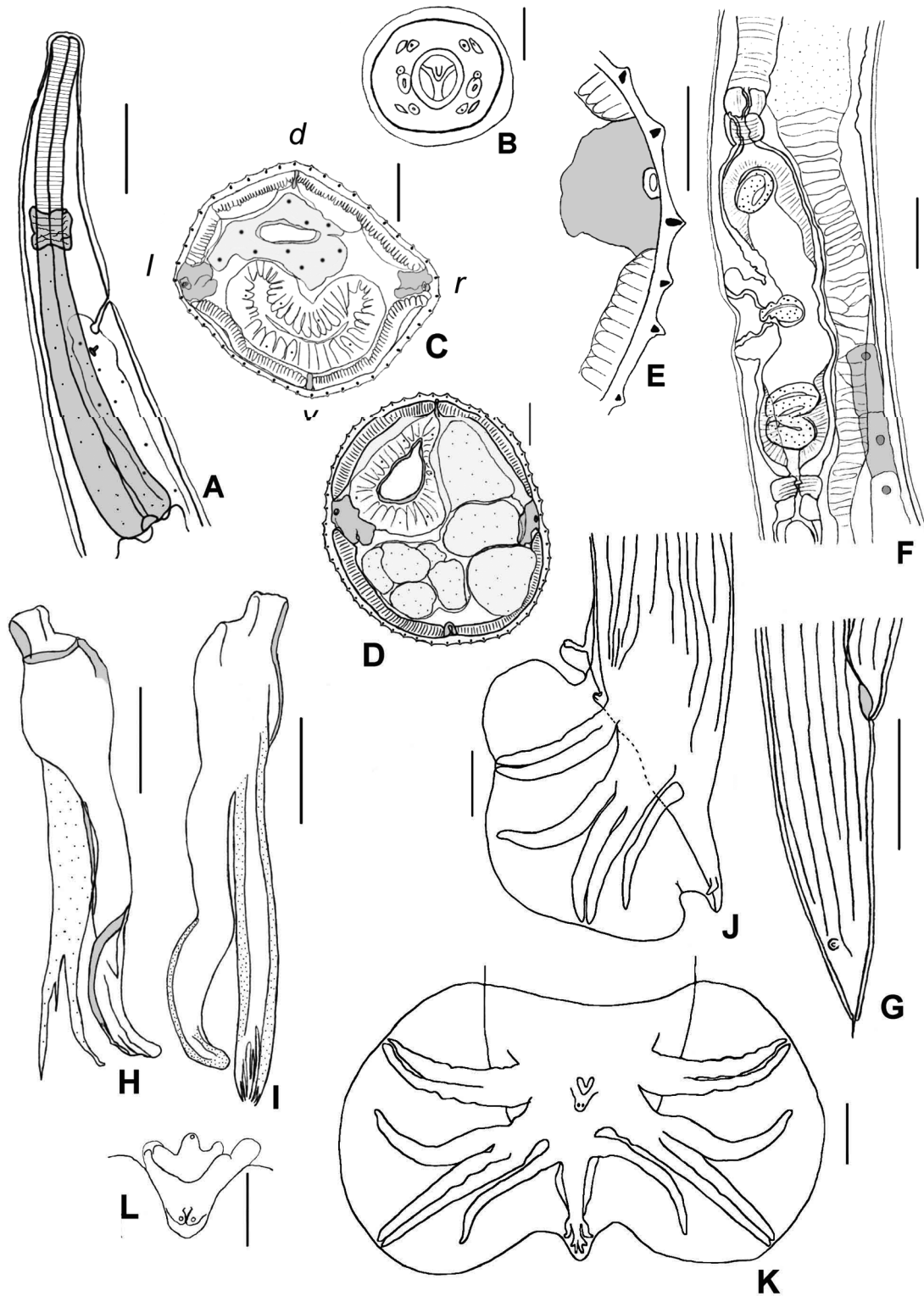


Fig. 2. A-L. *Oswaldocruzia urubambaensis* sp. nov.: **A.** Female, anterior end, right lateral view; **B.** Female, head apical view; **C.** Male, transverse section at mid-body; **D.** Female, transverse section at mid-body; **E.** Female, right cervical ala at level of excretory pore; **F.** Female, vulvar and ovejector regions; **G.** Female, tail, right lateral view; **H.** Male, right spicule, externo-dorsal view; **I.** Male, left spicule, sub-ventral view; **J.** Male, caudal bursa, lateral view; **K.** Male, caudal bursa, ventral view; **L.** Male, genital cone, ventral view. Scale bars: A, F-G = 100 µm; B, E, L = 20 µm; C, D, H-K = 50 µm

Spicules 201 (190–209) long, divided proximally, into three main branches, shoe, blade, distally divided into 6–9 small branches of unequal length, and fork divided at 28% of total length of spicule.

Female: (16 exemplars) 15.4 (12.0–18.4) mm long and 231 (204–302) wide at mid-body. Cephalic vesicle 87 (74–96) long and 50 (43–55) wide. Nerve ring, excretory pore, and deirids 237 (216–264), 390 (312–444), and 443 (372–497) from apex, respectively. Oesophagus 626 (588–677) long.

Didelphic, vulva at 5.4 (4.2–6.3) mm from caudal extremity. Vagina vera: 78 (62–108) long, dividing vestibule

436 (372–485) long into two equivalent parts. Sphincters both 42 (36–48) and infundibula both 34 (29–41) long, respectively. Uterine branches with more than 200 eggs. Many eggs embryonated 84 (79–91) long and 41 (36–46) wide. Tail 218 (156–269) long and 97 (72–113) wide at anus level with caudal spine 15 (11–19) long. Phasmids at 57 (53–62) from tip.

Host: *Rhinella marina* (Linnaeus, 1758) (Bufonidae).

Location: small intestine.

Locality: San Martin, North of Rio Camisea, Cusco, Peru (11°47'S, 72°47' W, 480 m).

Table I. Species of *Oswaldocruzia* in Bufonidae and names of host former in *Bufo*

<i>Oswaldocruzia</i> species	Original host	Actual name
<i>O. albaretii</i> Ben Slimane and Durette-Desset 1996a	<i>Bufo marinus</i>	<i>Rhinella marina</i> (Linnaeus, 1758)
<i>O. andersoni</i> Ben Slimane et Durette-Desset 1997	<i>Bufo americanus</i>	<i>Anaxyrus americanus</i> (Holbrook, 1836)
<i>O. arabica</i> Durette-Desset, Nasher and Ben Slimane 1992	<i>Bufo arabicus</i>	<i>Duttaphrynus arabicus</i> (Heyden, 1827)
<i>O. audebertae</i> Ben Slimane and Durette-Desset 1997	<i>Bufo americanus</i>	<i>Anaxyrus americanus</i> (Holbrook, 1836)
<i>O. barusi</i> Ben Slimane and Durette-Desset 1995b	<i>Bufo empusus</i>	<i>Peltophryne empusus</i> Cope, 1862
<i>O. belenensis</i> Santos, Giese, Maldonado and Lanfredi 2008	<i>Bufo marinus</i>	<i>Rhinella marina</i> (Linnaeus, 1758)
<i>O. bialata</i> (Molin, 1861)	<i>Bufo viridis</i>	<i>Pseudepidalea viridis</i> (Laurenti, 1768)
<i>O. canadensis</i> Ben Slimane and Durette-Desset 1997	<i>Bufo americanus</i>	<i>Anaxyrus americanus</i> (Holbrook, 1836)
<i>O. chambreri</i> Ben Slimane and Durette-Desset 1993	<i>Bufo typhonius</i>	<i>Rhinella margaritifera</i> (Laurenti, 1768)
<i>O. dlouhyi</i> Ben Slimane and Durette-Desset 1995a	<i>Bufo</i> sp.	<i>Rhinella</i> sp.
<i>O. filiformis</i> (Goeze 1782)	<i>Bufo viridis</i>	<i>Pseudepidalea viridis</i> (Laurenti, 1768)
<i>O. fulleborni</i> Ivanitsky 1940	<i>Bufo viridis</i>	<i>Pseudepidalea viridis</i> (Laurenti, 1768)
<i>O. galeanoae</i> Ben Slimane and Durette-Desset 1995c	<i>Bufo bufo</i>	<i>Bufo bufo</i> (Laurenti, 1768)
<i>O. gracilipes</i> Durette-Desset and Vaucher 1979	<i>Bufo gracilipes</i>	<i>Amietophrynus gracilipes</i> (Boulenger, 1899)
<i>O. hepari</i> Koo 1939	<i>Bufo melanostictus</i>	<i>Duttaphrynus melanostictus</i> (Schneider, 1799)
<i>O. hoepflii</i> Hsu 1935	<i>Bufo melanostictus</i>	<i>Duttaphrynus melanostictus</i> (Schneider, 1799)
<i>O. indica</i> Lal, 1944	<i>Bufo melanostictus</i>	<i>Duttaphrynus melanostictus</i> (Schneider, 1799)
<i>O. insulae</i> Morishita, 1926	<i>Bufo formosus</i>	<i>Bufo japonicus</i> Temminck et Schlegel, 1838
<i>O. iwanitskyi</i> Sudarikov 1951	<i>Bufo viridis</i>	<i>Pseudepidalea viridis</i> (Laurenti, 1768)
<i>O. kashmiriensis</i> Fotedar 1980	<i>Bufo viridis</i>	<i>Pseudepidalea viridis</i> (Laurenti, 1768)
<i>O. lescurei</i> Ben Slimane and Durette-Desset 1996a	<i>Bufo typhonius</i>	<i>Rhinella margaritifera</i> (Laurenti, 1768)
<i>O. mazzai</i> Travassos 1935	<i>Bufo marinus</i>	<i>Rhinella marina</i> (Linnaeus, 1758)
<i>O. melanosticti</i> Gupta 1960	<i>Bufo melanostictus</i>	<i>Duttaphrynus melanostictus</i> (Schneider, 1799)
<i>O. michaeli</i> Durette-Desset, Nasher and Ben Slimane 1992	<i>Bufo regularis</i>	<i>Amietophrynus regularis</i> (Reuss, 1833)
<i>O. mitunagai</i> Durette-Desset, Nasher and Ben Slimane 1992	<i>Bufo melanostictus</i>	<i>Duttaphrynus melanostictus</i> (Schneider, 1799)
<i>O. ohlerae</i> Ben Slimane and Durette-Desset 1996b	<i>Bufo camerounensis</i>	<i>Amietophrynus camerounensis</i> (Parker, 1936)
<i>O. perreti</i> Durette-Desset and Vaucher 1979	<i>Bufo latifrons</i>	<i>Amietophrynus latifrons</i> (Boulenger, 1900)
<i>O. polycercus</i> Baker 1982	<i>Bufo polycercus</i>	<i>Amietophrynus tuberosus</i> (Günter, 1858)
<i>O. proencai</i> Ben Slimane and Durette-Desset 1995a	<i>Bufo paracnemis</i>	<i>Rhinella schneideri</i> (Werner, 1894)
<i>O. stevensi</i> Ben Slimane and Durette-Desset 1997	<i>Bufo americanus</i>	<i>Anaxyrus americanus</i> (Holbrook, 1836)
<i>O. subauricularis</i> (Rudolphi, 1819)	<i>Bufo marinus</i>	<i>Rhinella marina</i> (Linnaeus, 1758)
<i>O. taranchoni</i> Ben Slimane and Durette-Desset 1995a	<i>Bufo marinus</i>	<i>Rhinella marina</i> (Linnaeus, 1758)
<i>O. tunisiensis</i> Durette-Desset, Nasher and Ben Slimane 1992	<i>Bufo viridis</i>	<i>Pseudepidalea viridis</i> (Laurenti, 1768)
<i>O. ukrainae</i> Ivanitsky, 1940	<i>Bufo viridis</i>	<i>Pseudepidalea viridis</i> (Laurenti, 1768)
<i>O. venezuelensis</i> Ben Slimane, Guerrero and Durette-Desset 1996	<i>Bufo marinus</i>	<i>Rhinella marina</i> (Linnaeus, 1758)
<i>O. yueni</i> Durette-Desset, Nasher and Ben Slimane 1992	<i>Bufo melanostictus</i>	<i>Duttaphrynus melanostictus</i> (Schneider, 1799)

Types: Holotype male (CP-MBUCV 6100), allotype female (CP-MBUCV 6101). Paratypes 2 males and 3 females (CP-MBUCV 6102), 2 males and 2 females (MNHN 648 MQ), host N° 020-010597.

Other material: Locality: Cashiriari, South of Rio Camisea, Cusco, Peru (11°53'S, 72°39'W, 690 m).

Two males and 3 females (CP-MBUCV 6103), host N° 148-010597; 3 males and 4 females (CP-MBUCV 6104), same data but host N° 149-010597.

Etymology: Toponymic derived from the main river in the region of collection (Rio Urubamba).

Discussion: The species belong to the Neotropical Continental Group of Ben Slimane, Durette-Desset and Chabaud (1996) with caudal bursa of type I, there are 5 species in it: *O. brasiliensis* Lent et Freitas, 1935 a parasite of Colubridae and Gekkonidae from Brazil, *O. lopesi* Freitas et Lent, 1938, a parasite of Leptodactylidae from Brazil, *O. neghmei* Puga, 1981 a parasite of Leptodactylidae from Chile, *O. vitti* Bursey et Goldberg, 2004, a parasite of Gymnophthalmidae from Brazil, and *O. costaricensis* Bursey et Goldberg, 2005 a parasite of Ranidae from Costa Rica. *O. neghmei* has the origin of rays 9 arising at mid-length of dorsal trunk, not at its distal extremity as in *O. urubambaensis*. *O. costaricensis* and *O. vitti* have not reinforcements in the cuticular ridges of the synlophe, the new species has. *O. lopesi* and *O. brasiliensis* have shorts spicules, 126–139 and 112–135, respectively, both have well developed cuticular cephalic vesicle, rays 8 arising not perpendicularly to dorsal trunk and rays 4 shorter than the couple 2–3, *O. urubambaensis* has longer spicules (190–209), a thin cephalic vesicle, rays 8 arising perpendicularly to dorsal trunk and rays 4 and 2–3 of the same length.

General Discussion

So far, 36 species of *Oswaldocruzia* have been reported around the world parasitizing the genus *Bufo* (s.l.), in Table I the nomenclature of the hosts according to Frost (2011) has been updated. In the Neotropics there are now 14 species parasitizing the genus *Rhinella*, 8 of which are in *Rhinella marina* (L., 1758), the species of greater distribution is widespread from the center of South America to South Texas, the Southeast Asian and Australia (Maciel 2008). Similar to other bufonids such as the black-spined toad, *Duttaphrynus melanostictus* (Schneider, 1799), widespread throughout in southern Asia, having 6 species of *Oswaldocruzia*, the European green toad, *Pseudepidalea viridis* (Laurenti, 1768), with 7 species distributed from France to Kazakhstan and the American toad, *Anaxyrus americanus* (Holbrook, 1836) in Canada to Northern Texas with 4 species, see Table I.

The presence of 2 species *O. manuensis* sp. nov. and *O. urubambaensis* sp. nov. in nearby localities in the eastern part of Peru is not surprising since *O. dlouhyi* Ben Slimane et Durette-Desset, 1995a and *O. subauricularis* (Rudolphi, 1819) have been found in the same individual host and *O. mazzai*

Travassos, 1935 and *O. subauricularis* (Rudolphi, 1819) have been found in nearby localities in Ecuador (Ben Slimane and Durette-Desset 1993), and 2 distinct karyotypes of *Rhinella marina* were found in East Peru (Cordova and Descailleaux 1996). The great biodiversity and distribution of the genus *Rhinella* make it possible to some undescribed species of *Oswaldocruzia* may be found, most of them very closely related, and gene sequencing in combination with morphological studies is necessary to decipher the diversity and evolution of this genus.

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